strain may include H44/76 HOPS-D strain (B1), 8570 HOS-G1 strain (B2), and/or B16B6 HPS-G_2A strain (B3).

[0011] In yet another aspect, the present technology provides a genetically modified vaccine strain of *Neisseria meningitidis* subtype B derived from: H44/76 strain comprising the genetic modifications of i) inactivation of a synX gene, ii) inactivation of the lpxL1 gene, iii) inactivation of the lgtA gene, iv) insertion of a second porA gene in the place of a opaD gene, v) increased expression of NadA compared with the native strain, and yl) stabilized increased expression of Opc and PorA proteins. In some aspects, the genetically modified strain was derived from the ET-5 wild type strain H44/76 (B:15: P1.7,16: L,3,7:P5.5,C).

[0012] In another aspect, the present technology provides a genetically modified vaccine strain of *Neisseria meningitidis* subtype B strain: derived from 8570 comprising the genetic modifications of: i) inactivation of a synX gene, ii) inactivation of the lpxL1 gene, iii) inactivation of the lgtA gene, iv) insertion of a second porA gene in place of opaD; v) increased expression of factor H binding protein variant 1; and yl) stabilized increased expression of PorA and Opc proteins. In some aspects, the genetically modified strain was derived from the ET-5 wild type strain 85 70(B:4: P1.19,15: L3,7v: P5.5,11,C).

[0013] In yet another aspect, the present technology provides a genetically modified vaccine strain of *Neisseria meningitidis* subtype B derived from B16B6 comprising the genetic modifications of: i) inactivation of a synX gene, ii) inactivation of the lpxL1 gene, iii) inactivation of the lgtA gene, iv) insertion of a second porA gene (subtype P1.22-1,4) in place of opaD; v) increased expression of factor H binding protein variant 2; and yl) stabilized increased expression of PorA and Opc proteins. In some aspects, the genetically modified strain is derived from the ET-37 wild type strain B16B6 (B:2a:P 1.5,2: L2:P5.1,2,5).

[0014] In some aspects, the present technology provides a genetically modified strain grown in iron deficient medium.

[0015] In other aspects, the present technology provides a genetically modified strain wherein inactivation of synX gene, lpxL1 gene, or lgtA gene is by an insertion of a drug resistance gene within the sequence of the inactivated gene.

[0016] Yet another aspect provides a vaccine including NOMVs derived from the genetically modified strains of the present technology. The NOMV are prepared from packed cells or spent culture medium without exposure to a detergent or denaturing solvent. The vaccine may further comprise one or more adjuvants. In further aspects, the genetically altered strain is altered to express iron uptake proteins.

[0017] In a further aspect, the present technology provides a vaccine against meningococcal disease comprising a variety of native outer membrane vesicles (NOMVs), wherein at least some of the NOMVs are essentially free of expression or sialylation of lipooligosaccharide (LOS), contain LOS that includes a lipid A with a penta-acyle structure and contain increased expression levels of at least one minor conserved outer membrane protein, wherein the minor conserved outer membrane protein is selected from proteins that induce bactericidal antibodies. The minor conserved outer membrane protein can be selected from the group consisting of NadA, factor H binding protein (FHBP) variant 1, and FHBP variant 2. In other aspects, at least some of the NOMV comprise shortened or truncated LOS that are essentially free of lacto-N-neotetraose (LNnT) tetrasaccharide and/or at least some of the NOMV comprise two or more different PorA proteins.

[0018] In another aspect, the present technology provides a method of eliciting an immune response to meningococcal disease in an animal or human comprising administering the composition containing NOMVs from at least one genetically altered strain of *N. Meningiitdis* to the animal or human for immunization against meningococcal disease. The vaccine is used for immunization against group B meningococcal disease.

[0019] In a further aspect, the present technology provides a method of preparing a genetically modified strain of *N. meningitidis* for use in a vaccine against meningococcal disease comprising the steps of: a) selecting a strain of meningococcal type B able to be genetically modified; b) genetically modifying the strain by inactivating the synX gene, c) genetically modifying the strain by inactivating the lpxL1 gene, d) genetically modifying the strain by inactivating the lgtA gene, and e) genetically modifying the strain by increasing expression of one or more minor conserved outer membrane proteins. In further aspects, the method further comprises genetically modifying the strain by inserting at least one second antigenically different porA gene into the open reading frame of the opa gene. In other aspects, the method further comprises the step of genetically modifying the strain to stably express or over express at least one outer membrane protein by replacing the poly-C sequence within the promoter or open reading frame of the at least one outer membrane protein with a sequence containing G and C nucleotides.

[0020] In yet another aspect, the present technology provides a method of preparing a vaccine against meningococcal disease comprising the steps of: a) culturing a genetically modified strain of *N. meningitidis* comprising one or more modification selected from the group consisting of inactivation of the synX gene, inactivation of the lpxL1 gene, inactivation of the IgtA gene, insertion of at least one second antigenically different porA gene in place of the opa gene, increased or stable expression of at least one minor conserved outer membrane protein, and/or stabilized expression of at least one outer membrane protein; b) expanding the culture by fermentation using the cultured strain of a) to inoculate medium in a fermentor; c) inactivating the fermented culture; d) harvesting *N. meningitidis* cultured cells by continuous flow centrifugation and collecting cell paste; e) isolating NOMVs from the cell paste; and f) resuspending NOMVs in buffer or carrier suitable for vaccine administration.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

[0021] FIG. **1** is a flow chart depicting the preparation of a master cell bank of cells for the genetically modified strains of *Neisseria* for vaccine production.

[0022] FIG. **2** is a flow chart depicting the production of the cell bank preparation used for making the genetically modified strains of *Neisseria* for vaccine production.

[0023] FIG. **3** is a flow chart depicting the fermentation of the *Neisseria* used for making the genetically modified strains of *Neisseria* for vaccine production.

[0024] FIG. **4** is a flow chart depicting the purification of NOMVs from the genetically modified strains of *Neisseria* for vaccine production.

[0025] FIG. **5** is a continuation of the flow chart from FIG. **4**.

[0026] FIG. **6** is a picture of a coomassie blue stained gel showing the protein content of standard marker (lane 1),